CIGUATERA AND HERBIVORES: UPTAKE AND ACCUMULATION OF CIGUATOXINS IN *CTENOCHAETUS STRIATUS* ON THE GREAT BARRIER REEF

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Ctenochaetus striatus is a common detritivorous grazer likely to be a key species transfering ciguatoxin precursors (gambiertoxins) to carnivorous reef fish. Toxins in tissues from *C. striatus* collected in the Great Barrier Reef were characterised by mouse bioassay and chromatography. The biodetritus on which it feeds were collected with an airlift suction apparatus and the toxins present compared with those in *C. striatus*. Toxins resembling gambiertoxins and ciguatoxins predominated in all samples. Lesser amounts of fast acting and unidentified toxins were also detected. Maitotoxin was not detected. Similar concentrations of the ciguatoxins and gambiertoxins were detected in *C. striatus* from John Brewer or Davies Reefs, despite the former having major crown of thorns starfish damage. Toxins detected in *C. striatus* from these reefs were below levels that would result in prey species becoming ciguateric. This assessment is consistent with the historically low risk of contracting ciguatera from carnivorous fish captured at these reefs. We were unable to detect any of the less-polarity gambiertoxins in the liver of *C. striatus*, suggesting that these toxins were biotransformed to the more polar ciguatoxins (ciguatoxin-1, -2 and/or -3) in the liver of herbivorous fish. The concentrations of ciguatoxin in the visceral contents of *C. striatus* were 3- to 6-fold lower than the levels of such toxins in the biodetritus, perhaps as a result of bacterial degradation associated with the active fermentation employed as part of the digestive strategy of this species. Alternatively, the gambiertoxins may have been rapidly assimilated from the intestinal contents of *C. striatus*, a feature that may explain the important role this species apparently plays as a vector for transfer of gambiertoxins (and ciguatoxins) to carnivorous fish.

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Surgeonfish (Acanthuridae) are common benthic herbivores on coral reefs world-wide. Especially common is *Ctenochaetus striatus*, a detritivorous grazer often suggested as a key species involved in the uptake and transfer of toxins involved in ciguatera (Randall,1958; Yasumoto et al., 1971; Banner,1984). *C. striatus* feeds by combing biodetritus from turf algae and coincidentally ingests a variety of toxin producing benthic dinoflagellates in the process. Of these dinoflagellates only *Gambierdiscus toxicus* has been confirmed to produce toxins which accumulate in fish and cause ciguatera (Yasumoto et al., 1977; Murata et al.,1990; Holmes et al., 1991; Lewis et al.,1991; Lewis & Sellin,1992).

Early studies suggested that *G. toxicus* produces one lipid-soluble toxin of similar polarity to ciguatoxin (Yasumoto et al.,1977); however, recent studies (Murata et al.,1990; Holmes et al.,1991; Holmes & Lewis,1992; Legrand et al.,1992) determined that *G. toxicus* produces several less-polar ciguatoxin precursors named gambiertoxins (a class of sodium channel activator toxins). These gambiertoxins apparently undergo oxidative metabolism, at some undefined point(s) in the food chain, giving rise to the various ciguatoxins including CTX-1, CTX-2 and CTX-3, the principal toxins found in the flesh and viscera of ciguateric carnivorous fish (Murata et al.,1990; Lewis et al., 1991; Lewis & Sellin,1992; Lewis et al.,1993). With this new understanding of the toxins involved in ciguatera, we have examined reef biodetritus and the visceral contents, viscera and liver of *C. striatus*. Comparison of the toxicity of *C. striatus* collected from the crown of thorns starfish damaged John Brewer reef and the lightly damaged Davies Reef allowed an assessment of the impact of such damage on toxin levels in fish.

METHODS

SAMPLE COLLECTION

Adult *C. striatus* were collected by spear from back reef areas of John Brewer Reef (18° 38'S, 147° 04'E) and Davies Reef (188 50'S, 147° 39'E). Specimens were collected in 2–5m during 9–10 December 1987 from two sites at John Brewer Reef (n=22 fish at each site) and from one site at Davies Reef (n=30 fish). Viscera and liver were removed soon after capture and visceral contents (including stomach contents) were separated from the viscera by carefully stripping them out. The remaining viscera was subsequently rinsed in seawater to remove any remnants of visceral contents. The visceral contents, viscera and liver were separately pooled for each site, and wet weights determined for each pooled sample. The samples were initially stored (4 days) in an equal volume of methanol (preservative) at 0–4°C. On return to the laboratory samples were stored at –20°C prior to extraction.

Biodetritus samples were taken at approximately 2m from John Brewer (site 2) and Davies Reefs. To mimic the feeding of *C. striatus*, we used an airlift suction apparatus fitted with a plastic bristled brush and powered by compressed air from a SCUBA tank. This allowed removal of biodetritus from the turf algae covering dead coral surfaces by a combination of scrubbing and suction actions. The turf algal areas sampled were typical of areas subject to the major feeding activity of *C. striatus*. Material from $0.8m^2$ of turf covered dead coral was collected into a floating plankton mesh sock (50μm mesh). The particulate material remaining in the sock was concentrated to a small volume, diluted 1:1 v/v with methanol and stored as for the *C. striatus* samples.

ISOLATION OF TOXINS

Samples were first freed of the methanol preservative before homogenisation in acetone (3x, 3:1 v/w). The dried extracts were then suspended in 90% methanol-water and the hexane- soluble material removed (3x, 1:1 v/v) by liquid-liquid partitioning. The 90% methanol-soluble material remaining was dried, suspended in water and extracted with diethyl ether (3x, 1:3 v/v). The ether-fraction was further separated into cold acetone-soluble and insoluble material following precipitation at –20°C. The acetone-solubles were further fractionated on silicic acid columns (100 mesh, Mallinkrodt using a minimum of 30 g silica/g extract) eluted with chloroform-methanol (c:m) mixtures of increasing polarity as described previously (Lewis et al.,1991). The water-soluble material from each site was extracted with n- butanol and pooled before further fractionation on a silicic acid column (Biosil A, Biorad) eluted with c:m mixtures as described by Holmes et al. (1990).

MOUSE BIOASSAY

Fractions were suspended in a 1% Tween 60/0.9% saline solution and bioassayed by intraperitoneal (i.p.) injection of Quackenbush strain mice (20± 2g, either sex, n=2–5). Signs of intoxication following injection were recorded to allow characterisation of the type of toxin present (ie. ciguatoxin, gambiertoxin, fast acting or undetermined). To avoid non-specific toxic effects ≤30mg of each fraction was injected per mouse. Fractions were designated as containing CTX-1 if bioassay signs of severe laboured respiration and loss of activity as well as at least diarrhoea, hypersalivation or lachrymation. Fractions were designated as containing GTX or less-polar ciguatoxins (e.g. CTX-2, CTX-3) if signs of hind-limb paralysis were observed in addition to the sign for CTX-1. Fast acting toxins were identified by injecting doses varying by 2- to 10-fold and recording time to death. Fast acting toxins typically caused deaths within an hour at doses approximating the minimum lethal dose. This protocol was sufficient to indicate such toxins had dose vs. time to death relationships clearly different from those for ciguatoxins, gambiertoxins or maitotoxins (Holmes et al.,1990,1991; Lewis et al.,1991,1992). Fractions designated as containing ciguatoxin or gambiertoxin on the basis of bioassay signs were quantified from the time to death relationship: $\log(\text{dose}) = 2.3 \log(1 + T^{-1})$, where dose is in mouse units (MU) and time to death (T) is in hr (Lewis et al.,1992). This approach allowed quantification and toxin characterisation with a minimum number of mice. Animal experiments were conducted in accordance with NHMRC animal ethics guidelines.

RESULTS

DIVER OBSERVATIONS ON *C. STRIATUS* FEEDING

C. striatus is the predominant grazing species at John Brewer and Davies reefs with most *C. striatus* feeding in shallower waters (1–5m). This herbivore was observed to feed throughout the day by combing biodetritus from turf algae covering the exposed dead coral surfaces. *C. striatus* was not observed to remove the turf algae in the

process of removing the biodetritus adhering to these algae. This was confirmed by a visual assessment of the gut contents.

G. TOXICUS COLLECTIONS AND TOXIN ANALYSIS

G. toxicus were found attached to numerous species of macroscopic algae sampled at John Brewer Reef (Table 1). *G. toxicus* were also a conspicuous component of the vacuumed biodetritus but the *G. toxicus* in these samples were not quantified. The biodetritus upon which *C. striatus* feeds and the visceral contents, viscera and liver of *C. striatus* were extracted and bioassayed for the presence of toxins. Table 2 indicates the toxins detected after liquid-liquid partitioning into hexane, ether-(acetone-soluble and -insoluble fractions) and butanol-soluble fractions. The less polar (hexane-soluble) material did not contain detectable toxicity, whereas the more polar fractions were often found to be toxic. The mouse bioassay detected gambiertoxin- and ciguatoxin-like toxins and several fast acting toxins in these more polar fractions.

The toxins in the acetone-soluble ether-fraction were further characterised by mouse bioassay after silica gel chromatography (Table 3). This allowed separation of (i) less polar toxins (GTX-4b-like) which elute with 97:3 c:m, (ii) toxins of similar polarity to CTX-1,-2 or -3 or the more polar gambiertoxins which elute with 9:1 c:m, and (iii) toxins with polarity similar to the maitotoxins which elute with 0:1 c:m (Murata et al.,1990; Holmes et al.,1990,1991; Lewis et al., 1991; Holmes & Lewis,1992). Toxins eluting with 97:3 or 9:1 c:m induced signs of toxicity characteristic of the ciguatoxins or gambiertoxins. The most polar toxins induced either CTX-1-like signs, signs of undetermined origin or were fast acting, with the toxicity varying between the sites and the samples investigated. The relative concentration of toxins (MU/g) in the 97:3 and 9:1 c:m fractions at each reef are compared in Fig. 1.

Toxins in the butanol extracts were also characterised after silicic acid chromatography (Table 4). After silica gel chromatography, toxins were found only in the c:m 9:1 and 0:1 eluates. The fast acting toxin from the visceral contents appeared to be less polar than the fast acting toxin in the detritus. Carry-over of ciguatoxins into the butanol fraction may explain the toxicity in the c:m 9:1 eluates. Toxins inducing signs characteristic of the maitotoxins were not detected in the

TABLE 1. Population densities of *Gambierdiscus toxicus* on macroalgae on John Brewer Reef (September, 1986)

Macroalgae	*G.toxicus*/100g macroalgae	Depth (m)	n
Spyridia filamentosa	3200	3	1
Padina australis	330-840	1 - 5	3
Halimeda opuntia	40 - 1000	2-10	4
H. incrassata	30	2	1
H. turia	30	3	1
Broodlea sp.	1120	3	1

TABLE 2. Yield (g) and characterisation * of lipid-soluble extracts from *Ctenochaetus striatus* and detritus, Great Barrier Reef.

	John Brewer Reef (1)			John Brewer Reef (2)				Davies Reef			
Fraction	Visceral Content (377g)	Viscera (200g)	Liver (100g)	Detritus (70g)	Visceral Content (434g)	Viscera (410g)	Liver (90g)	Detritus (150g)	Visceral Content (331g)	Viscera (380g)	Liver (130g)
Hexane	5.29	9.59	2.82	0.11	8.14	13.26	4.19	0.18	3.98	20.97	5.89
Acetone-soluble ether	0.62[b]	0.23b	1.11[d]	0.08[b]	0.78[b]	0.42[b]	1.41	0.13[b]	1.32[b]	0.41[b]	1.16[d]
Acetone-insoluble ether	0.05[b]	0.01[d]	0.63	0.01d	0.06[d]	0.03[b]	0.44[d]	0.02[b]	0.12b	0.05	0.84
Butanol	2.30[c]	0.91[d]	0.59[d]	0.23	3.15c	2.60[d]	0.92d	0.17c	2.30[d]	2.98	0.82

* Fractions were separated on the basis of solubility and those lethal to mice at ≤1.0g/kg were characterised as indicated by a-c below (n=2).

[a] Sign(s) in mice typical of ciguatoxin-1 (CTX-1) including lachrymation, hypersalivation and/or diarrhoea.

[b] Signs in mice typical of less polar ciguatoxins (CTX-2, CTX-3, GTX-4b), include those sign(s) induced by CTX-1 plus hind-limb paralysis.

[c] Fast acting toxin.

[d] Sign(s) of CTX-1 in mice for fractions non-lethal at 1.0g/kg.

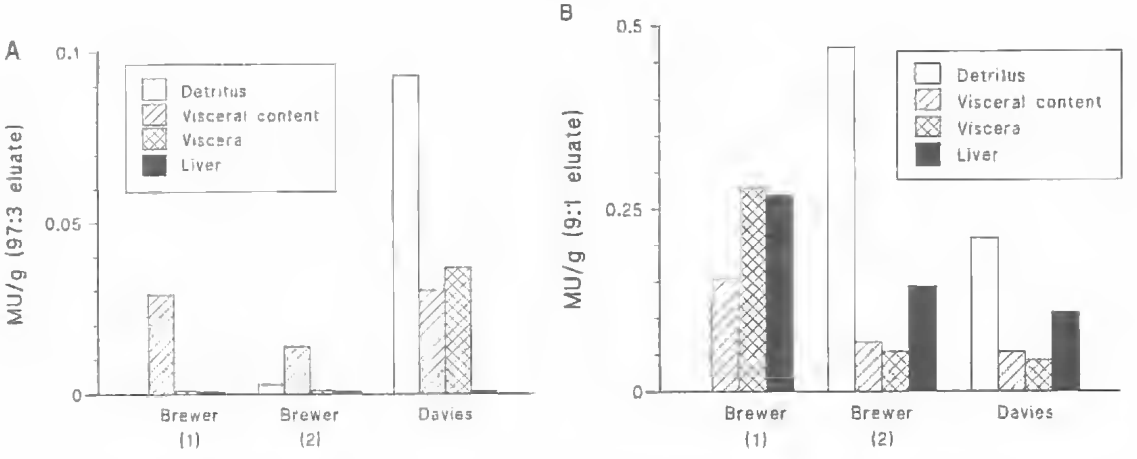

FIG. 1. Concentration of gambiertoxins and ciguatoxins in *Ctenochaetus striatus* and associated reef biodetritus. (A) Toxin levels in the 97:3 chloroform-methanol (c:m) eluates (low polarity gambiertoxins). (B) Toxin levels in the 9:1 c:m eluates (ciguatoxins and high polarity gambiertoxins). Toxin levels are given as total mouse units (MU)/g of sample. Cold acetone-soluble ether extracts were applied to silicic acid columns in each case.

butanol fractions, even after silica gel chromatography.

DISCUSSION

C. striatus feed by combing biodetritus from turf algae covering dead coral surfaces of these reefs (Purcell & Bellwood,1993). The turf algae (and other macroalgae) on these reefs were covered with moderate numbers of *G. toxicus*, indicating that turf algae is a niche in which *G. toxicus* might proliferate.

Several toxins were detected in extracts of the turf biodetritus and *C. striatus* visceral contents, viscera and liver. Ciguatoxin(s) and gambiertoxin(s) predominated in these samples which also contained several fast acting and several unidentified toxins. Interestingly, no maitotoxin was detected. Concentration of ciguatoxins and gambiertoxins in the tissues of *C. striatus* from

TABLE 3. Yield [g (MU)] and bioassay signs* of cold acetone-soluble ether-extracts separated by silicic acid chromatography

	John Brewer Reef (1)			John Brewer Reef (2)				Davies Reef			
Fraction[e] (c:m)	Visceral Content (0.5g)	Viscera (0.19g)	Liver (1.07g)	Detritus (0.06g)	Visceral Content (0.75g)	Viscera (0.38g)	Liver (1.37g)	Detritus (0.11g)	Visceral Content (1.29g)	Viscera (0.38g)	Liver (1.12g)
97:3	0.06 (11)[a]	*0.01 (0)	0.18 (0)	0.002 (0)	0.09 (6)c	0.03 (0)	0.14 (0)	0.01 (14)[b]	0.04 (10)[a]	0.08 (14)[b]	0.07 (0)
95:5	0.04 (3)[b]	0.01 (0)	0.15 (0)	*0.001 (20)[d]	0.07 (37)[b]	0.04 (0)	0.12 (0)	0.02 (0)	0.08 (4)[c]	0.06 (0)	0.12 (0)
9:1	0.37 (59)[b]	0.07 (56)[b]	0.28 (27)[b]	0.02 (33)[d]	0.44 (29)[b,c]	0.21 (22)[b]	0.56 (13)[a]	0.03 (32)[b]	0.53 (18)[b,c]	0.15 (16)[b]	0.22 (14)[a]
8:2	0.04 (2)[b,c]	0.02 (0)	0.002 (0)	0.001 (0)	0.05 (0)	0.03 (0)	0.04 (0)	0.01 (0)	0.18 (0)	0.02 (7)[d]	0.21 (0)
0:1	0.07 (3)[b,c]	0.03 (0)	0.07 (5)[b]	0.01 (0)	0.08 (4)c	0.04 (2)[c]	0.09 (0)	0.02 (20)[d]	0.38 (19)[d]	0.11 (0)	0.11 (0)

* Fractions lethal to mice at ≤1.0g/kg were quantified in mouse units (MU) as described in Methods (n=2-5 mice per fraction). The liver from Brewer (2) induced signs of ciguatoxin intoxication but no deaths (20mg was estimated to contain 0.5MU).

[a-c] Signs in mice as defined in Table 2.

[d] Signs in mice not clear.

[e] Fractions eluted from 100 mesh silicic acid with chloroform-methanol (c:m) mixtures of increasing polarity.

TABLE 4. Yield (g) and bioassay signs* of butanol extracts[e] separated by silicic acid chromatography

Fraction[f] (c:m)	Detritus (0.8g)	Visceral Content (2.5g)	Viscera (2.0g)	Liver (2.2g)
1:0	0.001	0.01	0.006	0.006
97:3	0.002	0.05	0.02	0.001[b]
9:1	0.006[d]	0.74[c]	0.07[b]	0.07[d]
0:1	0.11[c]	1.30	1.00	1.80

* Fractions lethal to mice at ≤1.5g/kg were characterised (n=2).

[a-d] Signs in mice as defined in Table 3.

[e] Butanol extracts (g) were pooled for the three sites, except for the detritus fraction which was from the Davies Reef collection only.

[f] Fractions eluted from Biosil A silica gel with chloroform-methanol (c:m) mixtures of increasing polarity.

John Brewer and Davies Reefs were similar, despite evidence of major damage at the former reef as a result of a crown of thorns starfish infestation. Our sample areas were surveyed in 1985 and had live:dead coral ratios of 1:3 and 2:0 for John Brewer and Davies Reefs, respectively (The Crown-of-Thorns Study,1985). If the area of turf algae is a factor that limits *C. striatus* density in reef areas, increased areas of dead coral would allow larger areas of turf algae which could support higher densities of *C. striatus*. These higher herbivore densities might in turn increase the proportion of such herbivores in the diet of carnivorous fish. This scenario could result in an increase in the rate carnivorous fish accumulate ciguatoxins. Increased areas of turf algae would also reduce the feeding pressure on turf algae which could possibly favour higher densities of *G. toxicus* on turf alae. Environmental factors, as yet unidentified, may also increase the levels of gambiertoxins per unit area of turf algae and such factors are perhaps more important in increasing the rate at which gambiertoxins and ciguatoxins enter the food chain of fish in coral reef areas.

The ciguatoxins in fish are believed to arise through the biotransformation (oxidative metabolism) of gambiertoxins produced by *G. toxicus* (Murata et al.,1990; Holmes et al.,1991; Holmes & Lewis,1992). Since low polarity gambiertoxins were not detected in *C. striatus* liver, we propose that the liver is a site, perhaps the major site, for biotransformation of the gambiertoxins in this species. Concentrations of ciguatoxin-like toxins in the biodetritus were considerably less than (3- to 7-fold) the concentrations found in the visceral contents of *C. striatus*. *C. striatus* does not employ acid digestion (gut was found to have a pH = 6.4) but fermentation is an important step in the digestion and assimilation of biodetritus by *C. striatus* (H. Choat pers. comm.). Such a reduction in toxin levels between the biodetritus and the visceral contents may stem from microbial degradation of the gambiertoxins to less potent forms. Alternatively, the uptake of gambiertoxins from the visceral contents of *C. striatus* may be rapid. A rapid uptake of gambiertoxins by fish (and man) may be a feature common to this class of polyether toxins.

This study found that levels of gambiertoxins entering *C. striatus* were typically higher than levels in the liver of this species. Consequently the gambiertoxins and their biotransformed products (ciguatoxins) do not appear to be accumulated in a simple, additive manner, suggesting that depuration of ciguatoxins and/or gambiertoxins may be significant in *C. striatus*. Such depuration by herbivores could, at least in part, contribute to the rapid decline in the ciguatoxin levels in a population of moray eels (Lewis et al.,1992) and the rapid decline in ciguatera incidence in some Pacific Island countries (Lewis,1992). A similar conclusion can be drawn from the study of Bagnis et al. (1985) who showed that a decline in *G. toxicus* numbers paralleled the decline in *C. striatus* toxicity.

Fish sampled in this study had relatively low levels of ciguatoxins compared with *C. striatus* from French Polynesia (Yasumoto et al.,1971; Bagnis et al.,1985). To initiate a ciguatera outbreak at these locations on John Brewer and Davies Reefs, we suggest that orders of magnitude higher gambiertoxin production per unit area of turf algae are required. Assays with higher sensitivity and specificity for toxins involved (e.g. antibody-based assays selective for the different gambiertoxins and their metabolites) or sites harbouring more toxic fish are likely prerequisites to the further study of toxins in *C. striatus*.

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